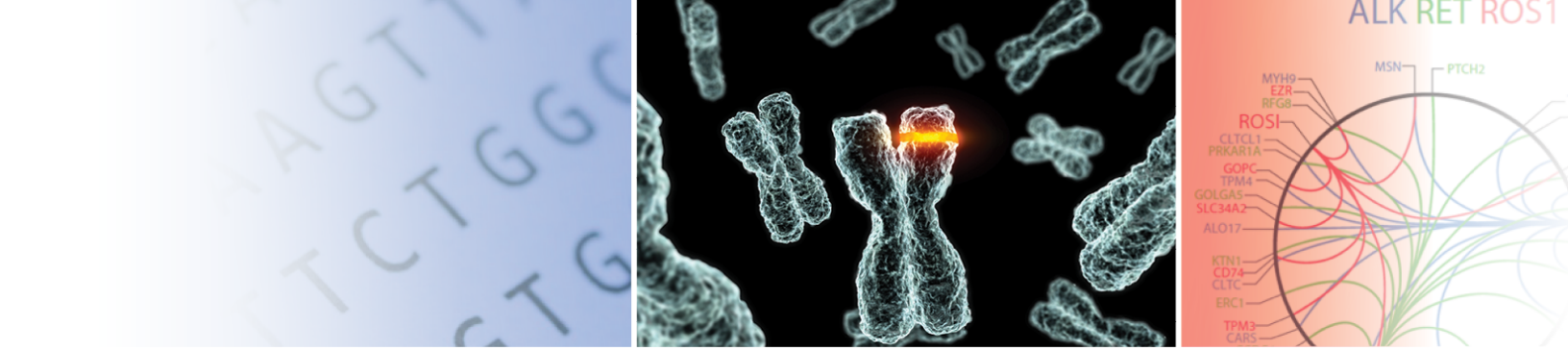



Instructions for Use

ARCHER™

# Archer™ Frequently Asked Questions

For products including:

| Catalog # | Description |
|---|---|
| AK0024-8 | Archer Universal RNA Reagent Kit v1 for Illumina® Platform |
| AK0040-8 | Archer Universal RNA Reagent Kit v2 for Illumina® -8 |
| AK0025-8 | Archer Universal RNA Reagent Kit v1 for Ion Torrent™ Platform |
| AK0042-8 | Archer Universal RNA Reagent Kit v2 for Ion Torrent™ -8 |
| AK0028-8 | Archer FusionPlex™ ALK, RET, ROS1 Panel v2 |
| AK0029-8 | Archer FusionPlex Heme Panel |
| AK0030-8 | Archer FusionPlex FGFR Panel |
| AK0031-8 | Archer FusionPlex NTRK Panel |
| AK0032-8 | Archer FusionPlex Sarcoma Panel |
| Variable | Archer FusionPlex Custom |

**Q: Which control genes are used in the Archer FusionPlex Assays?**

A: The control genes for the assays are CHMP2A, RABA7A, GPI and VCP. These are **housekeeping gen**es required for the maintenance of basic cellular function.

## Input Nucleic Acid Concentration and Purification

**Q: What types of starting material are recommended for the kits and how should they be processed?**

A:

| Starting Material | Recommended Extraction Method | Recommended Elution Buffer | Quantification Method | Recommended Quality Check Step |
|---|---|---|---|---|
| Formalin-fixed, paraffin-embedded (FFPE) tissue | Agencourt® FormaPure® Total Nucleic Acid Extraction (A33342) or QIAGEN® AllPrep DNA/RNA (80234) | 10mM Tris or nuclease-free water (No EDTA) | Qubit™ RNA HS Assay Kit (Life Technologies® Q32852) | Agilent© RNA 6000 Nano RNA Bioanalyzer Assay (5067-1511) |

| | | | | |
|---|---|---|---|---|
| Fresh frozen (FF) tissue, cell lines and blood | Any total RNA extraction kit | 10mM Tris or nuclease-free water (No EDTA) | Qubit RNA HS Assay Kit (Life Technologies® Q32852) | Agilent© RNA 6000 Nano RNA Bioanalyzer Assay (5067-1511) |

**Q: Do you have any suggestions for using the Agencourt FormaPure kit (A33342) when performing nucleic acid extraction of FFPE samples?**

A: We do have modifications to the published instructions.

- DO NOT treat the extracted total nucleic acid with DNase. Doing so will critically reduce the quality of RNA in the sample.
- Be sure to follow the total nucleic acid extraction workflow in the Agencourt® protocol, which does not include the DNase treatment.
- Use heat blocks versus water baths throughout the protocol.
- Perform an overnight digestion of FFPE samples at 55°C for 16 hours at Step 5 of the Agencourt protocol.
- Elute the sample in 40µL nuclease-free water at Step 23. This is the minimum elution volume.

See our modified FormaPure FFPE Sample Prep demonstration at http://archerdx.com/agencourt-formapure-ffpe-extraction-suggestions.html


**Q: Do you have any suggestions for using the QIAGEN All-Prep DNA/RNA FFPE kit (cat no 80234) when performing nucleic acid extraction of FFPE samples?**

A: We do have some suggestions:

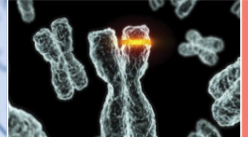
- Use QIAGEN Deparaffinization Solution (cat. no. 19093) instead of harsh chemical treatments such as Xylene.
- Do not treat the extracted total nucleic acid with DNase. Doing so may critically reduce the quality of RNA in the sample.
- Follow the RNA extraction protocol that DOES NOT include small RNAs.

**Q: If I DNAse-treated my FFPE sample during the extraction process, will the assay function correctly?**

A: DNAse treatment can reduce the quality of the RNA in the sample. When using FFPE samples, total nucleic acid is the recommended starting material.

**Q: What is the minimum amount of starting material for the assay?**

A: The minimum recommended input for the assay is 20ng total nucleic acid. The amount of input material can be adjusted according to the quality of the RNA sample. When using less than 10ng input material, the number of PCR cycles for First and Second PCR may need to be increased. Note that reduced sample input may adversely affect read diversity. Please refer to the following Instructions for Use:



| | |
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| AK0025-8 | Archer Universal RNA Reagent Kit for Ion Torrent™ Platform |
| AK0042-8 | Archer Universal RNA Reagent Kit v2 for Ion Torrent™ -8 |

**Q: What is the recommended maximum input amount for the kits?**

A: If higher library complexity is desired, the assay can tolerate up to 250ng total nucleic acid, too much input will decrease final library yield.

**Q: Can Tris-EDTA buffer be used instead of 10mM Tris-HCl pH 8.0 buffer to elute and store my samples?**

A: Do not use buffers with EDTA. Use nuclease-free water or 10mM Tris-HCl pH 8 for sample storage.

**Q: Can genomic DNA be used with this protocol?**

A: Not at the present time. Archer Universal RNA Reagent kits are designed for total nucleic acid or RNA as the starting material.

**Q: What should I do if my input sample is too dilute to run the Archer assay?**

A: Repeat the nucleic acid isolation to increase quantity of starting material available for the assay.

**Q: How can I check the quantity and quality of my RNA sample?**

A:

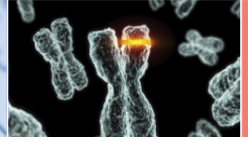
- Determine the sample concentration on the Qubit instrument using the HS RNA Assay Kit (Life Technologies Q32852).
- Visualize the size distribution and RNA integrity on an Agilent Bioanalyzer RNA 6000 Nano Kit (5067-1511)
- Universal RNA Kits version 2 (AK0040-8, AK0042-8) come with PreSeq™ qPCR QC to assess RNA quality and help predict whether or not there is sufficient good quality RNA to pass QC. PreSeq can also be purchased separately from the RNA assay if you would like to screen all RNA samples prior to proceeding through library preparation.

**Q: How does PreSeq work?**

A: PreSeq primers target a 113-bp region of VCP (one of the housekeeping control genes targeted in the Archer assay). The 113-bp region spans an exon-exon junction, so background DNA will not interfere with RNA quality assessment. A Ct cutoff is experimentally determined for all RNA samples above which samples will not pass QC on Archer Analysis.

**Q: What is the minimum RNA fragment size for starting material?**

A: The assay requires RNA fragments to be a minimum of 60 to 70 bases in length. The Universal RNA Reagent kit v2 (AK0040-8, AK0042-8) contains RNA QC primers that will amplify a 113-bp amplicon of the VCP gene. After first-strand cDNA synthesis, the sample can be tested by qPCR to determine whether to continue with the assay. Please refer to the Instructions for Use for more information.



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| AK0042-8 | Archer Universal RNA Reagent Kit v2 for Ion Torrent™ -8 |

## Kit Storage & General Protocol

**Q: What are the recommended kit storage conditions?**

A: Store all components of the Universal RNA Reagent kits at 4ºC. The liquid gene-specific primers (GSP1s and GSP2s) should be stored at -20ºC.

**Q: The Archer RNA Universal Reagent kit was accidentally stored at –20ºC. Can it still be used?**

A: Storage at 4ºC is ideal; however, accidental storage of the kit at –20ºC will not adversely affect kit performance. Bring to room temperature prior to use.

**Q: The kit was accidentally left out overnight on the bench top. Can it still be used?**

A: Yes, you can use the kit if it has been left out overnight at room temperature. However, the pouches must have remained sealed with the desiccant packs inside.

**Q: Are there stopping points in the protocol?**

A: There are four stopping points in the workflow:

1. After Second Strand cDNA Synthesis
2. After Adapter Ligation
3. After First PCR
4. After Second PCR

**Q: How long does the library preparation protocol take to perform without stopping?**

A: The library preparation protocol takes approximately 7-9 hours of total time, depending on the version of the kit used, and 2 hours of hands-on time to process 8 samples.

**Q: Are there any commercially available positive or negative controls for the Archer FusionPlex ALK, RET, ROS1 v2 fusion detection assay?**

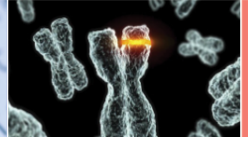
A: Yes, we recommend the following for positive and negative controls:

- Positive control – an ROS1-positive RNA sample, such as the HCC-78 cell line from Creative Bioarray© (CSC-C0569)
- Negative control – the Ambion® Human Lung Total RNA (Life Technologies AM7968)

**Q: Are there any commercially available positive or negative controls for the Archer FusionPlex Heme fusion detection assay?**

A: Yes, we recommend the following for positive and negative controls:

- Positive control – the High BCR-ABL p210 control that is part of the *ipsogen*® BCR-ABL Mbcr Control Kit (QIAGEN, 670191)



- Negative control – the Human Blood Leukocyte Total RNA (Ambion, R1234148-10)

**Q: Are there any commercially available positive or negative controls for the Archer FusionPlex FGFR fusion detection assay?**

A: Yes, we recommend the following for positive and negative controls:

- Positive control – the Negative BCR-ABL p210 & p190 that is part of the *ipsogen* BCR-ABL Mbcr Control Kit (QIAGEN, 670191) is positive for the FGFR1-FGFR1OP2 fusion.
- Negative control – the Human Normal Thyroid Total RNA (Ambion, R1234265-50)

## Sample Multiplexing, Adapter Ligation & First/Second PCR Steps

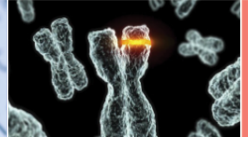
**Q: What are the part numbers for the Molecular Barcode Adapters used in the Archer FusionPlex Assays? Please, click on the links in the list below for more details regarding the MBCs or http://archerdx.com/mbc-adapters**

| Catalog # | Molecular Barcode Adapter (MBC) Description |
|---|---|
| SA0027 | Archer Barcode Adapters 1-8 for Ion Torrent Platform |
| SA0028 | Archer Barcode Adapters 9-16 for Ion Torrent Platform |
| SA0029 | Archer Barcode Adapters 17-24 for Ion Torrent Platform |
| SA0030 | Archer Barcode Adapters 25-32 for Ion Torrent Platform |
| SA0031 | Archer Barcode Adapters 33-40 for Ion Torrent Platform |
| SA0032 | Archer Barcode Adapters 41-48 for Ion Torrent Platform |
| SA0040 | Archer MBC Adapters A1-A8 for Illumina |
| SA0041 | Archer MBC Adapters A9-A16 for Illumina |
| SA0042 | Archer MBC Adapters A17-A 24 for Illumina |
| SA0043 | Archer MBC Adapters A25-A32 for Illumina |
| SA0044 | Archer MBC Adapters A33-A40 for Illumina |
| SA0045 | Archer MBC Adapters A41-A48 for Illumina |
| AK0016-48 | Archer MBC Adapters Set B for Illumina |
| AK0017-48 | Archer MBC Adapters Set C for Illumina |

**Q: What is the best way to use indexes for multiplexing samples?**

A: See the "Sample Multiplexing" section of the appropriate Archer Universal RNA Reagent Kit Instructions for Use for details. Select the document below for all relevant information.

| | |
|---|---|
| AK0024-8 | Archer Universal RNA Reagent Kit for Illumina® Platform |
| AK0040-8 | Archer Universal RNA Reagent Kit v2 for Illumina® -8 |
| AK0025-8 | Archer Universal RNA Reagent Kit for Ion Torrent™ Platform |
| AK0042-8 | Archer Universal RNA Reagent Kit v2 for Ion Torrent™ -8 |



**Q: How many samples can be multiplexed together?**

A: The level of multiplexing depends on the number of targets and the number of reads generated by the instrument per run. This will vary for each catalog panel as well as custom panels. Custom fusion detection assays will need to be optimized to balance the number of reads needed against the level of multiplexing.

| Archer Illumina Panel | # of Targets/Assay | Recommended # of Reads |
|---|---|---|
| FusionPlex ALK, RET, ROS1 Panel v2 | 29 | 300,000-500,00 |
| FusionPlex Heme Panel | 132 | 1,000,000-5,000,000 |
| FusionPlex Sarcoma Panel | 134 | 1,000,000-5,000,000 |
| FusionPlex NTRK Panel | 17 | 300,000-500,00 |
| FusionPlex FGFR Panel | 17 | 300,000-500,00 |

| Archer Ion Torrent Panel | # of Targets/Assay | Recommended # of samples/318 Chip |
|---|---|---|
| FusionPlex ALK, RET, ROS1 Panel v2 | 29 | 3-4 |
| FusionPlex Heme Panel | 132 | 2-3 |
| FusionPlex Sarcoma Panel | 134 | 2-3 |
| FusionPlex NTRK Panel | 17 | 7-10 |
| FusionPlex FGFR Panel | 17 | 7-10 |

**Q: Does Agencourt AMPure purification affect the complexity of my prepared libraries?**

A: No, the AMPure XP beads are designed to efficiently remove smaller molecules such as unincorporated dNTPs, primers, primer dimers, salts and other contaminants.

**Q: Will AMPure XP bead carryover impact any downstream steps?**

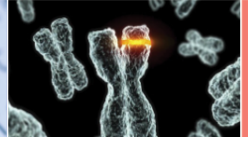
A: Remove all beads if possible. However, a very minimal amount of AMPure beads has no noticeable impact on the assay.

**Q: Why was a new polymerase (Phoenix Taq) chosen for PCR 1 and 2?**

A: ArcherDX is committed to continuously improving our product line for both the chemistry and software. The Phoenix Taq enzyme results in less mis-priming, leading to more reads from the correct targets or less false positive results.

**Q: Why does the Universal RNA Kit v1 have a ramp rate from 98ºC to 68ºC while the Universal RNA Kit v2 have a ramp rate from 95ºC to 65ºC?**

A: The ramp rates were optimized for each version of the kit. The RNA Kit v1 and v2 have different ramp rates, because different enzymes are used for PCR. The RNA Kit v1 uses VeraSeq Taq for PCR and the RNA Kit v2



uses Phoenix Taq for PCR. The modified ramp rate in the v2 kit is vital to the assay's function due to the complex nature of the Anchored Multiplexed PCR reaction. It is important to verify that this ramp rate is set correctly before starting the PCR program by consulting the instrument's user manual or contacting the manufacturer. Please see the table below for v2 kit ramp rates for specific thermal cyclers:

| Company | Thermal Cycler Model | Block Type | Maximum Block Ramp Rate (º/s) | Notes |
|---|---|---|---|---|
| Applied Biosystems® | GeneAmp® PCR System 9700 | 60 wells | 1.9 | |
| | | 96-Aluminum | 2.3 | |
| | | 96-Silver/Gold | 2.6 | |
| | | 2 x 96 | 1.6 | |
| | | 3 x 384 | 1.8 | |
| Life Technologies | Veriti® | Standard 96 | 3.9 | |
| | | Fast 96 | 5.0 | |
| | | 60 wells | 3.3 | |
| Bio-Rad | T100™ | 96 wells | 4.0 | Set ramp % under the denaturing step of the amplification cycle; can only be set at 0.2ºC or 0.3ºC/s. Default to 0.2ºC/s in this case |
| | C1000™ | 96 wells; 96 wells deep block; 2 x 48 block; 384 well block | 5.0 | |
| | S1000™ | 96 wells; 96 wells deep block; 384 well block | 5.0 | |
| Eppendorf | Mastercycler® Pro | 96-Aluminum | 4.0/3.0 | Block ramp rate – (heating ramp rate/cooling ramp rate) |
| | Mastercycler Pro 5 | 96-Silver | 6.0/4.5 | Block ramp rate – (heating ramp rate/cooling ramp rate) |
| | Mastercycler Pro 384 | Aluminum | 4.0/3.0 | Block ramp rate – (heating ramp rate/cooling ramp rate) |

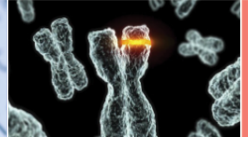
**Q: Is the modified ramp rate in either the 68ºC or 65ºC PCR step meant for ramping toward that temperature or away from it?**

A: Ramping towards that temperature. Therefore, the ramp rate should be set at either 2.3ºC/second moving down from 98ºC to 68ºC in Universal RNA Reagent Kit v1 (AK0024-8, AK0025-8) or at 0.28ºC/second moving down from 95ºC to 65ºC in the Universal RNA Reagent Kit v2 (AK0040-8, AK0042-8).

Ramp rates, Universal RNA Reagent Kit v1

PCR 1 conditions, heated lid — Volume = 20µL

| Incubation temperature | Incubation time | # cycles |
|---|---|---|
| 98ºC | 30 s | 1 |
| 98ºC | 10 s | 20* |
| 68ºC (ramp rate of 2.3ºC/s) | 30 s | |
| 72ºC | 3 min | 1 |
| 4ºC | HOLD | 1 |



* Adjustable

PCR 2 conditions, heated lid — Volume = 20µL

| Incubation temperature | Incubation time | # of cycles |
|---|---|---|
| 98ºC | 30 s | 1 |
| 98ºC | 10 s | 24* |
| 68ºC (ramp rate of 2.3ºC/s) | 30 s | |
| 72ºC | 3 min | 1 |
| 4ºC | HOLD | 1 |

* Adjustable

Ramp rates, Universal RNA Reagent Kit v2

PCR 1 conditions, heated lid — Volume = 20µL

| Incubation temperature | Incubation time | # cycles |
|---|---|---|
| 95ºC | 3 min | 1 |
| 95ºC | 30 s | 20* |
| 65ºC (ramp rate of 0.28ºC/s) | 60 s | |
| 72ºC | 3 min | 1 |
| 4ºC | HOLD | 1 |

* Adjustable

PCR 2 conditions, heated lid — Volume = 20µL

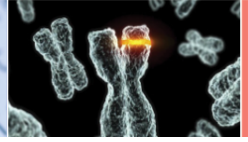
| Incubation temperature | Incubation time | # of cycles |
|---|---|---|
| 95ºC | 3 min | 1 |
| 95ºC | 30 s | 20* |
| 65ºC (ramp rate of 0.28ºC/s) | 60 s | |
| 72ºC | 3 min | 1 |
| 4ºC | HOLD | 1 |

* Adjustable

## Quantify Library and Sequence

**Q: Can a Qubit instrument be used to measure the concentrations of the final libraries instead of a KAPA Biosystems® Library Quantification Kit?**

A: Using the Qubit instrument is not recommended for the final library concentration. We cannot guarantee consistent loading concentrations with the Qubit, because a size selection of the final library is not performed. Therefore, we recommend using the appropriate KAPA Biosystems Library Quantification Kit for accurate quantification of sequenceable molecules. Other commercially available qPCR-based library quantification kits can also be utilized. Please refer to the Instructions for Use below for guidance:



| | |
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**Q: What is the average amplicon length for a FusionPlex assay?**

A: The expected average size for amplicons will range between 150 and 400 base pairs as viewed on a Bioanalyzer trace. However, you should assume an average fragment length of 250 base pairs when using the KAPA Biosystems Library Quantification Kit for qPCR. Our recommended dilutions and MiSeq® and PGM® input amounts are all based on an assumed average fragment length of 250 base pairs.

Q: What is the expected concentration of my final library?

A: Final library concentration should range between 50 and 500nM for the Universal RNA Reagent Kit v1 and 15 to 100nM for the Universal RNA Reagent Kit v2. Do not use libraries with concentrations below 2nM.

**Q: Why is my final library concentration low?**

A: Reasons for low final library concentrations include:

1. The source of the nucleic acid, the purification method, the chemical the nucleic acid is suspended in and the starting amount of nucleic acid can contribute to low library concentration. Extract RNA from FFPE tissue with one of our recommended methods. Make sure RNA input quantity was measured using the HS RNA Qubit assay and sufficient starting material was used. Additionally the sample should pass the PreSeq VCP qPCR QC step, which can be performed after first-strand cDNA synthesis to determine whether to continue with the assay or repeat the nucleic acid extraction and start over. Refer to the protocol (link to the protocol) for more information on the PreSeq RNA QC step.

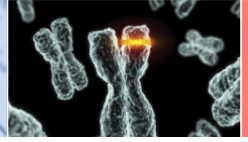
2. Incorrect PCR cycling can lead to low library yields. Ensure that the cycling temperatures, cycle numbers and ramp rates are accurate. Check the instrument to make sure it is calibrated and in good working order.

**Q: My template negative control produced sequenceable library. Is this normal?**

A: Yes, a template negative control will fail the QC filter in Archer Analysis and thus will not produce meaningful results.

**Q: Are MiSeq libraries compatible with the HiSeq® and NextSeq® instruments?**

A: Yes, as long as they are run on a paired-end flowcell using Nextera® chemistry. Please note that our libraries are dual indexed and should be sequenced using 2x150bp PE reads and 2x8bp index reads. For Nextera, you will need to add BP13 primers (included in the kit). Refer to the Instructions for Use below for guidance:



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| AK0042-8 | Archer Universal RNA Reagent Kit v2 for Ion Torrent™ -8 |

**Q: How much PhiX should I add to my library for the Illumina instrument platforms?**

A: Add 10% PhiX to the library to serve as a control on the MiSeq and add 30% PhiX on the NextSeq.

**Q: What cluster density can be expected on the MiSeq?**

A: This will vary by kit version. We recommend using the MiSeq Reagents Kit V2, 300 cycle (MS-102-2002). The typical cluster density with the recommended loading concentration of 10pM will be approximately ≥700 k/mm$^2$.

# Archer Analysis

## Software Installation

**Q: Where can I find the instructions to install and run Archer Analysis?**

A: The complete Installation Guide and Instructions for Use can be downloaded from the following link: http://archerdx.com/assets/documents/Archer-Analysis-Manual.pdf. Additionally, informational videos and links to other supporting materials can be found at http://archerdx.com/videos.

**Q: How much does the software cost?**

A: Nothing. Customers using any of the Archer panels can create an account and download the software for free. Visit archerdx.com/analysis for complete information regarding Archer Analysis.

**Q: Do I need to download the virtual machine or command line version of the software to try it?**

**A:** Users are not required to use either of these approaches. ArcherDX provides a free web-based version of Archer Analysis. Data on this server is not guaranteed to be secure or private and is primarily meant for demonstration purposes. Customers may also access a set of demo data using the link and login information below:

http://archerdx.com/demo-data
User Name: example@example.com
Password: password123

NOTE: Data loaded on this server will be removed after 30 days.

**Q: What are the requirements for the virtual machine and command line versions of Archer Analysis?**

A: Users must have a virtualization application, such as Virtual Box or VMWare installed on their system first. The system must have at least 12GB free RAM (total system RAM 16GB) and ~200GB available storage. Download the analysis package from http://archerdx.com/analysis. This package contains all of the analysis functionality in a single package that does not require any extra software (other than Virtual

Machine software). The command line version is provided for customers that would like to integrate Archer Analysis with their own analysis pipeline.

**Q: What virtualization software can be used to run the virtual machine version of Archer Analysis?**

A: We have extensively tested with Oracle Virtual Box (for workstations) and VMware vSphere (for larger installations), but other virtualization software should work equally well. Oracle VirtualBox can be downloaded using this link: https://www.virtualbox.org/

**Q: How much free memory should my machine have to run the virtual machine version of Archer Analysis?**

A: The system with the virtual machine will need at least 12 GB of free memory (RAM). This will allow a user to run a single sample at a time. More memory (12G more per sample) will allow users to run multiple samples at a time.

**Q: What web browser(s) are supported with Archer Analysis?**

A: While most current browsers are supported, we recommend using Chrome or FireFox. Note that Internet Older versions of Internet Explorer do not support multiple selections of FASTQ files in the file dialog box; however, most recent versions of Internet Explorer on work correctly.

**Q: Can I keep using the old version of Archer Analysis after you release a new version?**

A: Yes, this is one of the benefits of using virtual machines. You can have a virtual machine with each version of the software to allow for continuity and ease of validation

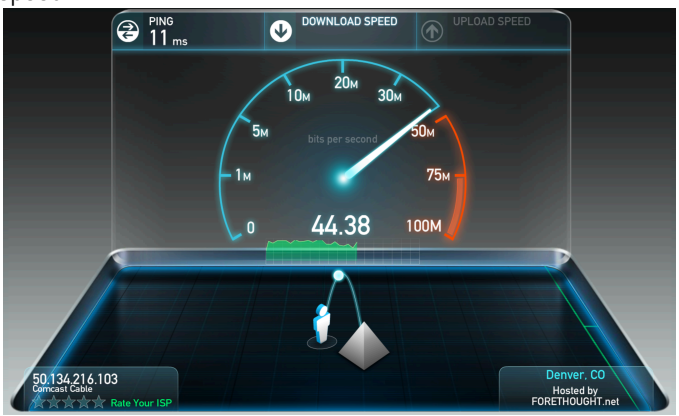
## Archer Analysis: Getting Started

**Q: What is the best way to upload files to Archer Analysis?**

A: We recommend that you put all of the FASTQ files to be uploaded into a single location. Note that all of the files for a single job must be uploaded at the same time. Make sure to select and upload both the read 1 and the read 2 FASTQ files for each sample when working with the Illumina® paired-end reads libraries.

**Q: How long will it take to upload my files?**

A: This will depends on your internet speed and file size. You can check your upload speed at http://speedtest.net and calculate your upload time by dividing the sum of your file size by your upload speed.



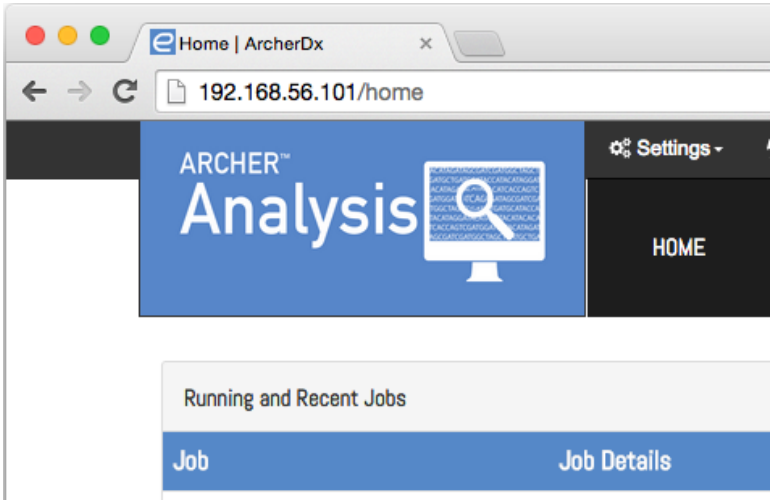

In this example, the speed is 44.38 megabytes/second. You would divide your file size by this number.

**Q: How long does Archer Analysis take to analyze data?**

A: The analysis of a single set of FASTQ files (~250,000 reads, or 50-100MB data) should take only a few minutes. Larger runs of multiple samples can take a few hours, depending on the complexity and amount of reads. By default, the VM runs each sample serially, but the system can be set up to process multiple samples at the same time. Consult the Instructions for Use about how to set up Archer Analysis for parallel processing of samples. The Archer Analysis Instructions for Use are located at http://archerdx.com/assets/documents/Archer-Analysis-Manual.pdf

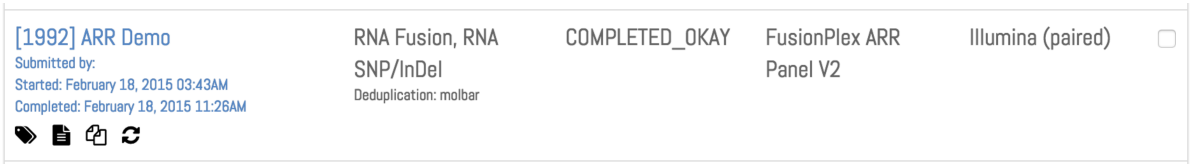
**Q: I installed Archer Analysis on my virtual machine, but it requires me to open my browser to run it. Am I online?**

A: No, you are not. The virtual machine generates a local IP address that you copy into your browser; however, you are only running on the local machine, not online. In the example below, the virtual machine is running on the IP address 192.168.56.101, which is internal to the organization and does not exist outside the firewall of the organization.

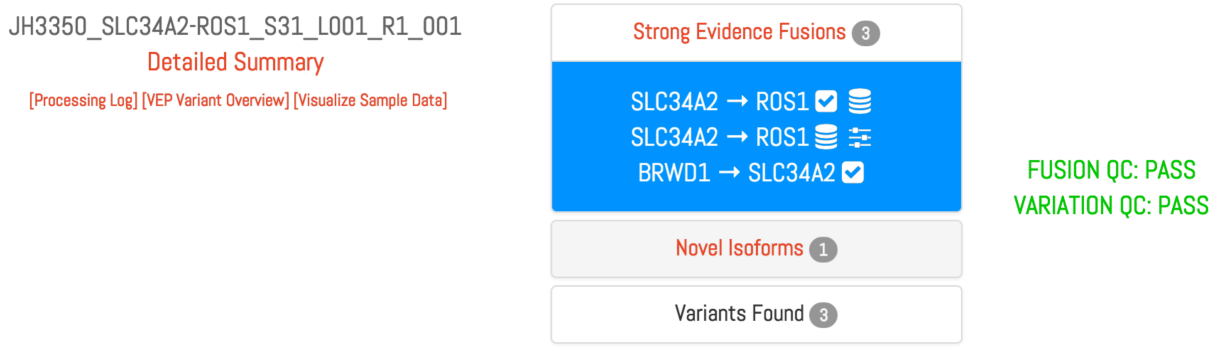



**Q: How do I know when my run is complete?**

A: Open the Archer Analysis home page on your virtual machine browser and check the "Job Status" beside your run. If the run is complete, you can click on the detailed summary of the run to see sample data. As shown in the example below:



**Q: What are the meanings of the symbols on the summary and evidence pages?**
**A:**




Means that the fusion passed all QC Metrics

Means that the fusion is a known fusion according to the Archer Quiver™ database

Means that the percent fusion reads for the GSP2 detecting that fusion is below the set threshold

Means that the fusion partners show sequence similarity to each other and could therefore be a mispriming event

Means that the fusion is an exon/intron fusion. Most fusions are exon/exon fusion

**Q: How is my data kept confidential?**
A: To ensure sample confidentiality, be sure to run Archer Analysis on a local machine and not the web-based option. This keeps your data on your local system, not the web.

## Archer Analysis: Expected Metrics

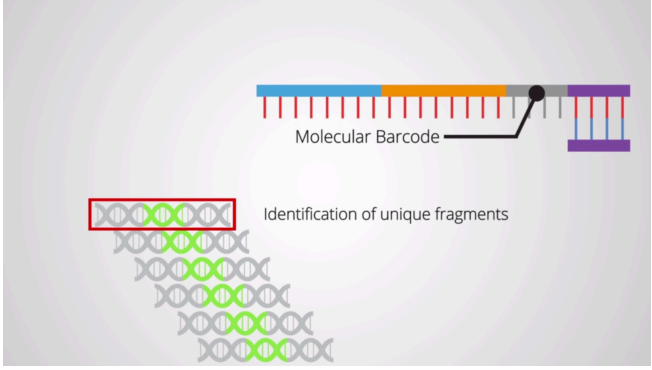
**Q: How many unique fragments (reads) do I need per sample?**
A: The number of fragments needed depends on the number of targets in your assay and the quality of your sample. The default cutoff is 200 unique fragments per target (GSP2). You can calculate the number of unique RNA reads (on target) you need to pass our standard QC cutoff by multiplying the number of targets in your panel by 200. This cutoff should be evaluated during validation and set appropriately for each lab.

**Q: What if I do not have 200 unique RNA reads per GSP2?**
**A:** Unless you have updated your analysis settings, your sample will not show "PASS" in the QC section. However, depending on your samples, the data may be fine, especially if the number is close to 200. Any positives detected are real if they meet all the other cutoffs. The minimum number of unique reads is set to ensure there are no false negatives. This is the reason the cutoff should be set by each lab during validation.

**Q: What is a molecular barcode (MBC)?**

A: A molecular barcode is a unique 8-bp sequence added to the molecule to identify unique fragments and "de-duplicate" the sequencing reads from a sample. This, along with the random start sites, helps identify and remove PCR duplicates.

Learn more about the value of MBCs for accurate data analysis. Watch the videos at http://archerdx.com/mbc-adapters.




**Q: What percent of my total fragments can I expect to have a molecular barcode?**

A: This percentage varies with library quality. For high quality libraries, the fragments with molecular barcodes should be 80-90% of the total fragments.

**Q: What if I have a very low number of fragments with molecular barcodes?**

A: This reduces the total number of fragments that are analyzed and may cause the sample to not pass QC but does not necessarily mean the run or results are bad. This can potentially be overcome by performing deeper sequencing.

**Q: What percent of my fragments with a molecular barcode can I expect to be unique?**

A: For good libraries with the correct amount of starting material, up to 20% of the fragments with molecular barcodes should be unique fragments. For FFPE samples, the percentage of unique fragments can drop to 1-10%. However, this is not a firm cutoff and varies with library quality and panel.

**Q: What does the On Target percent mean?**

A: On Target percent is the percentage of library fragments that include the entire GSP2, which requires that the fragment be long enough to reach the GSP2 and be of high enough quality at the end of that read to identify all bases in the GSP2.

**Q: Under the Read Statistics tab of the Detailed Summary page, how do you define a DNA vs. RNA vs. ambiguous read?**

A:

- A DNA read is a read that spans an intron/exon junction.
- An RNA read is a read that spans an exon/exon junction.
- An ambiguous read is a read that does not span any junction and therefore cannot be identified as DNA or RNA.

Q: How is the mapped fragment number calculated? (Under the Read Statistics tab of the Detailed Summary page)

Read Statistics — ROS-100ng_S81_L001_R1_001.combined

| Type | Total Fragments (#) | Mapped (# / %) | Pass Alignment Score Filter (%) | On Target (%) |
|---|---|---|---|---|
| All Fragments | 728,933 | 727,366 / 99.79 | 99.8 | 99.5 |
| Unique Fragments | 84,621 | 83,419 / 98.58 | 98.6 | 97.39 |

A: Archer Analysis utilizes BWA and Bowtie 2 for mapping. Any read that has an Alignment Score less than 30 is removed from consideration. Reads with a mapping quality less than 30 are either multiple mapping reads or contain many low-quality bases (low FASTQ base quality). For the exact calculation of the mapping quality and the factors involved, please refer to the BWA and Bowtie 2 documentation. Note that this default value can be adjusted by the user in the Analysis Settings area of Archer Analysis.

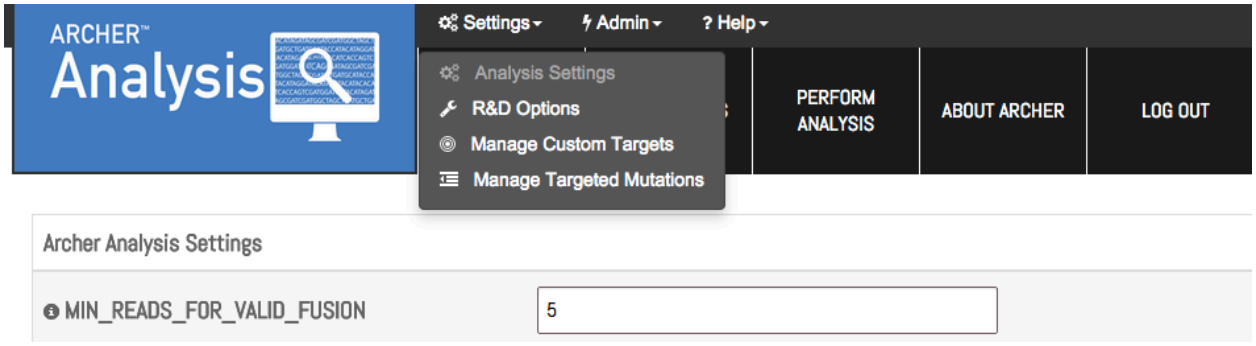
**BWA:** http://bio-bwa.sourceforge.net/bwa.shtml
**Bowtie:** http://bowtie-bio.sourceforge.net/index.shtml

Q: What are the criteria for the QC Result?

A: A sample will pass the QC filter if the number of unique (de-duplicated) RNA reads per target (Gene Specific Primer 2; GSP2) is at least 200. This has proven to be a reasonable criterion for the success of the assay. However, users are encouraged to evaluate the results themselves and set their own cutoff.

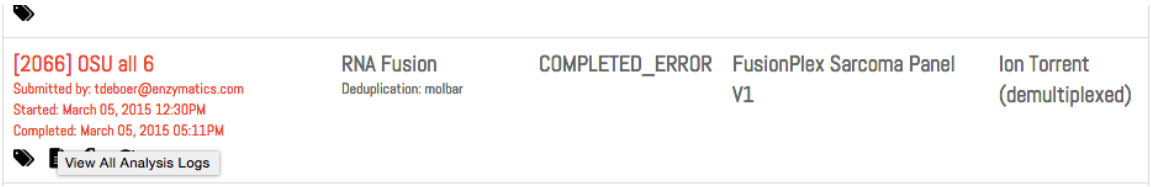
Q: What are the criteria for fusion candidates?

A: Fusion candidates are required to have a minimum of 5 unique (de-duplicated) break point-spanning reads that support the gene fusion. This is the default value; however, users can adjust this default value in the Analysis Settings area of Archer Analysis. They must also comprise at least 10% of the total reads for that GSP2, not be intron-exon fusions and not show any similarity to each other to be considered a "Strong Fusion" candidate. Fusions that are found in the Quiver™ database are always marked as "Strong Fusion" candidates, regardless of any of the other filters.




## Archer Analysis: Troubleshooting

Q: My job status says "COMPLETE_ERROR". What should I do?



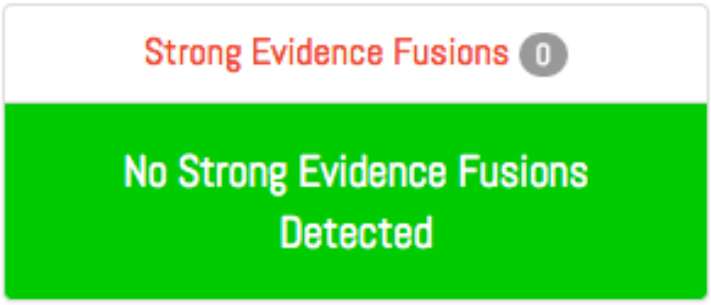

A: Make sure that you have:

1. Selected the correct de-duplication method
2. Selected the correct platform option (Illumina paired-end is the default)

If all these are correct, then contact Archer technical support at tech@archerdx.com or **877-771-1093.**

Consult the Analysis Logs by selecting the "View Analysis Logs" option ( ) to view the log files to obtain clues about why the error was produced. If possible, send the log files to ArcherDX technical support to help in the error analysis.

**Q: I chose the correct parameters, but my sample still failed QC, now what?**





A: This does not necessarily mean that your data is bad. Click on "Detailed Summary" to get more information about why it failed and decide if you want to accept the data, change parameters, or re-run the sample.

Read about what is acceptable data in these other FAQs:

1. What is the most common reason runs fail QC?
2. How do I know if my fusion is real?

**Q: What are the most common reasons runs fail QC?**

A: Run QC is most affected by starting library quality, fragment uniqueness and coverage. You will see the effects of these factors on "Average Unique Fragments per GSP2", because it takes many metrics into account. This is found under the Read Statistics tab in the 3rd set of boxes. The default setting is 200 Average Unique Fragments per GSP2 for RNA reads. You might want to change the 200 cutoff based on your validation results.

DNA/RNA Statistics — JH3343_EML4-ALK_S24_L001_R1_001

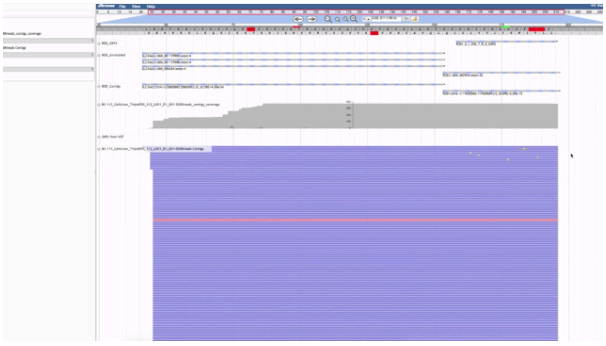
| Type | DNA Reads (# / %) | RNA Reads (# / %) | Ambiguous Reads (# / %) |
|---|---|---|---|
| All Fragments | 67,586.0 / 17.1 | 195,584.0 / 49.4 | 132,767.0 / 33.5 |
| Unique Fragments | 3,300.0 / 17.4 | 8,355.0 / 44.1 | 7,293.0 / 38.5 |
| Average Unique Fragments per GSP2 | 99.79 | 286.76 | 230.97 |

**Q: How do I know if my fusion is real?**

A: Archer has provided information to guide the detection of fusions and variations. However, every sample is different. Results must be interpreted by each lab and take into consideration any information know about their particular samples. Read statistics and the visualization tools can assist in making proper calls. Some things to consider include:

1. Does the fusion appear in the strong evidence tab?
2. Are there several reads supporting the fusion call? 5 is the MINIMUM number of unique reads to call a fusion
3. Does the fusion have a large percent of the reads as compared to the wild-type transcript?
4. Is the expression level of the target gene normally high or low?
5. How does the expression level of the target in the sample compare to normal?
6. Is the fusion known in the literature?
7. When visualizing the data, are there any insertions or deletions present in more than a few of the reads near the breakpoint? This could indicate a mapping error
8. When visualizing the data, do all of the reads have the same start or stop point? If so, they could be duplicates

Learn more about visualizing your fusions and reads in JBrowse. Watch the video at http://archerdx.com/support/faqs/archer-analysis-troubleshooting/how-do-I-know-if-my-fusion-is-real.



## Quiver

**Q: What is the Quiver Fusion Database?**

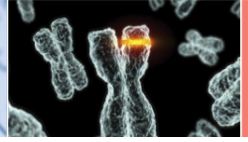
A: The Quiver Fusion Database is a compilation of known fusions from several public databases. Visit http://archerdx.com/quiver to learn more about it.

**Q: What can I use Quiver for?**

A: Quiver provides information on which of our panels include a particular gene or fusion, as well as which exons participate in the known fusion, and links to PubMed and other public resources. This is useful for researching fusions that you are unfamiliar with or when designing your own custom assay.

**Q: Can I add new fusions to Quiver?**

A: Currently you cannot, but we are working on adding that function. For now, if you find a novel fusion, contact tech support at tech@archerdx.com.



# Archer Custom Design Tools

## Assay Designer

**Q: What is Archer Assay Designer?**

A: Assay designer is our free online tool used to create your own custom assays. It can be found here: http://assay.archerdx.com/assay/.

**Q: How do I create a custom fusion panel?**

A: A custom fusion panel can be made at http://archerdx.com/assay-designer. Exons from genes can be selected and added to the custom fusion panel. It is also possible to upload a .csv file with the appropriate information. Once the design has been submitted, a representative from ArcherDX will contact you to make sure the design is correct. The Quiver Fusion database (http://archerdx.com/quiver) can also be used to find known fusions that can then be added to the custom design.

**Q: Why do I have less than 100% coverage of my target?**

A: Assay Designer might fail to design a primer to a target region for one or more of the following reasons:

1) Target region has high similarity to other regions in the genome, which would result in off-targeting
2) GC content in target region is too high or too low
3) There are known mutations present in the primer site

**Q: Can I adjust the primer search parameters to recover missed targets?**

A: No. The primer search setting in Assay Designer are fixed so that primers are optimal and likely to be fully functional with our assay. If you need assistance recovering important targets, contact technical support for assistance at tech@archerdx.com.

**Q: Are my primers tested and balanced before I order them?**

A: No. Primers are designed in silico and ordered directly through Assay Designer. They are not tested in any way prior to shipping. They will arrive in an equimolar pool. For best results, it is recommended that you test the primer pool on DNA before use for fusion detection. Testing the primers on DNA will allow you to confirm that all primers are fully functional without the added complication of expression levels associated with target amplification of RNA. If you are interested in having ArcherDx functionally test your primers and balance your primer pools, contact technical support at tech@archerdx.com.

**Q: What is the minimum number of targets I can include in an assay?**

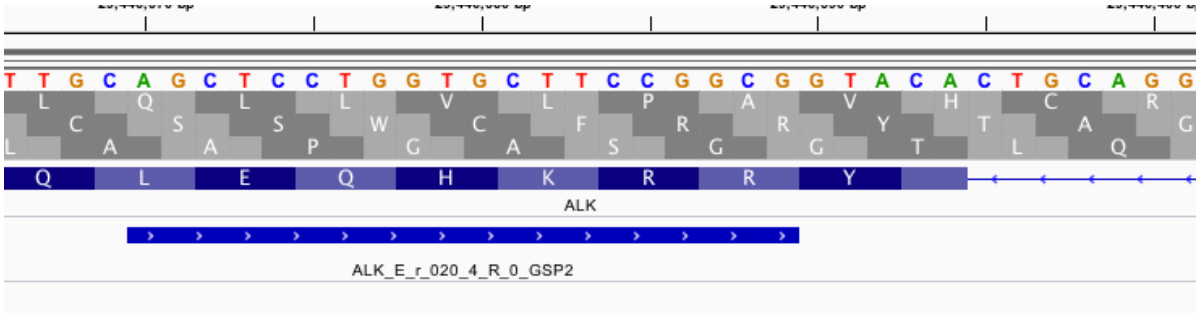
A: There is no current minimum. However, the standard 8 controls included in our catalog panels will be included in any design.

**Q: What is the maximum number of targets I can include in a panel?**

A: There is no specific maximum number of targets for Assay Designer. However, we will flag panel designs that we do not believe *in silco* design is sufficient to meet your expectations or ours. In this instance we will be in contact with you to discuss specifics and how you would best like to proceed with procuring a custom panel.

**Q: How long will it take to receive my custom primer order after submitting my order?**

A: After the design has been approved and submitted for order, you can expect to receive your equimolar primer pools in 6-8 weeks.




**Q: Why do my primers have to be reviewed before I can place my order?**

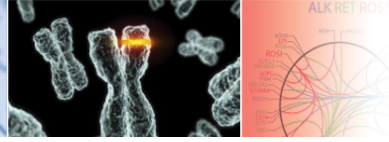
A: To provide you with the best chance of creating a successful primer design for your assay, a member or our team will carefully review each primer in your design and remove any primers expected to perform poorly. In some cases, we might recommend that the custom panel be redesigned by our in-house R&D team to ensure that they meet both your and our expectations.

**Q: Do I need to specify a breakpoint in order to detect fusion?**

A: Assay Designer assumes that all breakpoints are at the specified end of the exon (3′ or 5′). If you want to detect mid-exon breakpoint fusions, contact technical support at tech@archerdx.com.

**Q: Do I need to specify both fusion partners in order to detect fusions?**

A: No. ArcherDx's Anchored Multiplex PCR (AMP) chemistry enables you to detect any fusion partner to your target of interest. You do, however, need to specify the exon of interest for each target as well as the direction of the fusion partner from the target exon (3′ or 5′). Note that mid-exon breakpoint primer design is not supported by Assay Designer at this time. If you want to detect mid-exon breakpoint fusions, contact technical support at tech@archerdx.com.



For further assistance, please contact Archer Technical Support:

Email: *tech@archerdx.com*

Phone: 877-771-1093 or 303-357-9001

## Limitations of Use

**For Research Use Only.** Not for use in diagnostic procedures.

This product was developed, manufactured, and sold for in vitro use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.



For more information please visit http://www.archerdx.com

ArcherDX, Inc.
2477 55th Street, Suite 202
Boulder, CO 80301
303-357-9001
www.archerdx.com